Synthesis of Some Dehydrophenylalanine Peptides[1]

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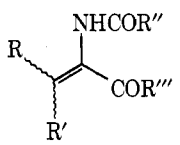
The bromo pseudoazlactones, **3**, were found to dehydrobrominate readily giving the unsaturated azlactones, **4**. These compounds, generated in situ, were converted into *N*-trifluoroacetyldehydro amino acid anilides (**5**) and peptides (**6**, **7** and **10**) and perhydro-1,4-thiazepin-5-ones (**11**). The trifluoroacetyl group was removed from the dehydrophenylalanine peptides in good yields. *N*-Trifluoroacetyldehydrovaline, isoleucine, leucine, alanine, and α-aminobutyric acid anilides and peptides were not deblocked by gaseous ammonia. The mechanism of the deblocking reaction is discussed.

A great deal of interest has been generated recently[3] in the synthesis of dehydro amino acids (DHA) and peptides (DHP) and their derivatives. DHA appear in numerous peptides of microbial origin[4] and are thought to be biological intermediates in the synthesis of important biologically active compounds such as penicillin.[5]

Several approaches to the synthesis of DHA have been developed. One method[6] depends on the elimination of the elements of water from β-hydroxyamino acids, while a second more general approach[7] uses pyrolytic elimination of an α,β-sulfoxido group to introduce the double bond. The first method is limited by the availability of β-hydroxyamino acids. The second requires the synthesis of the amino acid carbon chain by condensation reactions which often afford low yields especially when polyfunctional amino acids are involved. Our approach[8] to the synthesis of DHA has been that of direct introduction of the double bond into the intact amino acid chain by the oxidation of amino acid azlactones.[9] Our earlier work[8a] led to the synthesis of DHA derivatives in which an α-methylcinnamoyl group [**1**, R″ = $C_6H_5CH{=}C(CH_3)$–] remained on the nitrogen atom after introduction of the double bond. Hydrolytic removal of the



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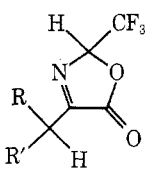
N-acyl group led, of course, to hydrolysis of the resulting enamine giving the α-keto acid. Clearly, if **1** could be prepared such that the *N*-acyl group were removable under *non*hydrolytic conditions, the free enamino acid could be obtained. This report describes in detail our more recent efforts[8b] to use the trifluoroacetyl group in the synthesis of **1** (R″ = CF_3) and our studies related to its nonhydrolytic removal.

Some years ago, Weygand reported[10] that amino acids could be converted into "pseudo" azlactones (**2**) by heating with trifluoroacetic anhydride and that these were readily brominated α to the azomethine function giving **3**. We found that when compounds of type **3** (Table I) were treated with *exactly*[11] 1 equiv of triethylamine, the unsaturated azlactones (**4**) were formed in excellent yield. The pseudo-



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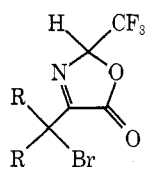
a, R = Ph; R′ = H
b, R = CH_3; R′ = CH_3
c, R = *i*-Pr; R′ = H



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d, R = C_2H_5; R′ = CH_3
e, R = R′ = H
f, R = CH_3; R′ = H

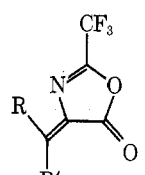

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Table I

Compd	R	R′	% yield	Bp, °C (mm)
3a	Ph	H	62	102–103[a] (CCl_4)[b]
3c	$(CH_3)_2CH$	H	86	91–94 (0.4)
3d	C_2H_5	CH_3	78	87–91 (12)

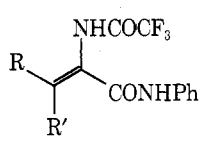
[a] Melting point. [b] Recrystallization solvent.

Table II

Compd	R	R′	% yield	Mp, °C
5a	Ph	H	83	191.5–193
5b	CH_3	CH_3	81	227–229
5c	$(CH_3)_2CH$	H	74	195–196
5d	C_2H_5	CH_3	64	220–222
5e	H	H	46	124–126

azlactones derived from alanine (**2e**) and butyrine (**2f**) showed a tendency to dibrominate,[10] but careful avoidance of excess bromine and fractional distillation of the bromo compounds allowed their isolation in acceptable yields. In this investigation, only the phenylalanine azlactone (**4a**) was isolated and its chemistry studied carefully. All of the other unsaturated azlactones were prepared and allowed to react in situ with aniline or an amino ester to form the isolated products.

The anilides (**5a–e**) could be prepared from the required bromo compounds (**3**) by treatment with 1 mol of triethylamine followed by 1 mol of aniline or, more conveniently, with 2 equiv of aniline alone. The anilides were crystalline compounds, easily characterized, and are described in Table II. When the azlactones (**4a–d**) were allowed to react with various amino esters, the dehydro dipeptide esters (**6a–m**) (Table III) were formed in 40–80% yields. One



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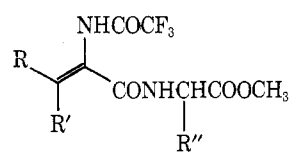

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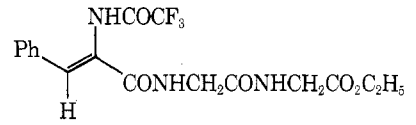

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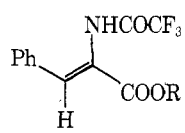

8a, R = H
b, R = CH_3

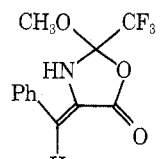

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Table III

Compd	R	R′	R″	% yield	Mp, °C (solvent)
6a	Ph	H	$PhCH_2$	76	143.5–144 ($CHCl_3$)
6b	Ph	H	$(CH_3)_2CHCH_2$	48	173.5–175 (CH_3O–H_2O)
6c	Ph	H	Ph	67	117–119 (Et_2O–ether)
6d	Ph	H	CH_2OH	81.5	169–171 (CH_3OH–H_2O)
6e	CH_3	CH_3	$PhCH_2$	70	143.5–145 (CH_3OH–H_2O)
6f	CH_3	CH_3	$(CH_3)_2CHCH_2$	62	158–162 (C_2H_5OH–H_2O)
6g	CH_3	CH_3	Ph	71	173.5–176 (CH_3OH–H_2O)
6h	$(CH_3)_2CH$	H	$PhCH_2$	57	87–90 (CH_3OH–H_2O)
6i	$(CH_3)_2CH$	H	$(CH_3)_2CHCH_2$	41	126–128 (CH_3OH–H_2O)
6j	$(CH_3)_2CH$	H	CH_2OH	47	97–99 (CH_3OH–H_2O)
6k	C_2H_5	CH_3	$PhCH_2$	50	131–136 (CH_3OH–H_2O)
6l	C_2H_5	CH_3	$(CH_3)_2CHCH_2$	58	139–146 (CH_3OH–H_2O)
6m[a]	Ph	H	H	76	118–120 (C_2H_5OH–H_2O)
6n[b]	Ph	H	H	73	165–168, 225–230 dec (CH_3OH–H_2O)
6p[c]	Ph	H	$PhCH_2$	26	182–188 (CH_3OH–H_2O)
10b	CH_3	CH_3	CH_2SH	45	181–184
10d	C_2H_5	CH_3	CH_2SH	38	183–187 (CH_3OH–H_2O)

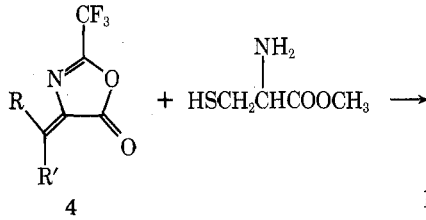
[a] Prepared as the ethyl ester. [b] Prepared as the amide. [c] Prepared as the acid.

Table IV

Compd	R	R′	% yield	Mp, °C (solvent)
11a	Ph	H	36	197–199 (MeOH–H_2O)
11c	$(CH_3)_2CH$	H	32	197–199.5 (CH_3OH–H_2O)
11e	H	H	41	236–240 (THF)
11f	CH_3	H	49	242–245 (CH_3OH)

dehydro tripeptide (**7**) was also prepared from glycylglycine ethyl ester in 86% yield. The excellent yield of this compound showed clearly that the rate of the bimolecular reaction between **4a** and the dipeptide ester, generated in situ from the hydrochloride, was sufficiently great to compete favorably with the rate of unimolecular diketopiperazine formation from the free dipeptide ester. The rate of hydrolysis of **4a** in aqueous acetone forming the acid (**8a**) was also considerably greater that of other unsaturated azlactones which we have studied in earlier work.[8a] The presence of the trifluoromethyl group in the 2 position of the azlactone ring apparently enhanced the reactivity of the 5-carbonyl function toward nucleophiles. Surprisingly, when **4a** was allowed to react with excess methanol, a complex mixture was formed. A crystalline product, isolated in only 28% yield, was finally assigned the structure **9** based on the NH (3340 cm^{-1}) and carbonyl (1795 cm^{-1}) absorptions in the infrared spectrum and the methoxyl protons in the ^1H NMR spectrum at δ 3.43 ppm. The fact that the CF_3 peak appeared as a singlet in the ^{19}F NMR spectrum confirmed this structure. The methyl ester, prepared by the treatment of **8a** with diazomethane, was different from **9** and had the expected physical properties. The azlactone **4a** was also allowed to react with an aqueous solution of sodium phenylalaninate to form, after acidification, the dehydro dipeptide acid **5p** in only 26% yield. Concurrent hydrolysis of the azlactone may have been responsible for the low yield.

Some very interesting results were obtained when several of the azlactones (**4a–f**), formed in situ, were allowed to react with cysteine methyl ester. Azlactones **4a,c,e,f** gave perhydro-1,4-thiazepin-5-ones (**11**) (Table IV) while **4b** and **4d** gave the unsaturated peptides (**10b,d**) only. These results indicated that ring closure occurred only when R′ was hydrogen and that the presence of even a methyl group at the R′ position was sufficient to prevent it. This result indicates that cysteinyldehydrovaline peptides, postulated as intermediates in the biosynthesis of penicillin[5], would not ring close spontaneously to thiazepinones during penicillin biosynthesis.[12] The facile ring closure of **10e,f** are examples of the type of Michael addition proposed by Gross[13] in the biogenesis of lanthionine and cyclolanthionine and reported by Zervas[6] in a recent synthesis of these compounds. Perhydro-1,4-thiazepin-5-ones of the type **11**,



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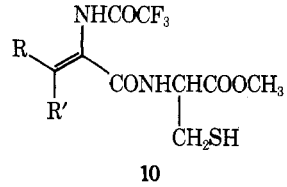

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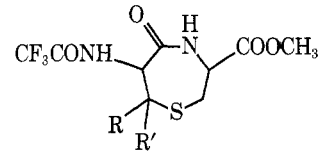

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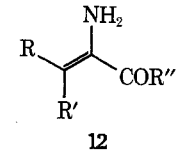
formed enzymatically, might be converted[14a,b] to penicillin by a transannular ring closure across the 4 and 7 positions, although this has not been accomplished chemically.[14c] Our method gives easy access to many different 7-substituted thiazepinones for further investigation of this possibility. The thiazepinones, isolated in 30–50% yields, obtained by our method were of unknown stereochemistry. Since the physical data indicated that they were most probably single entities, they probably represent the most stable isomer since they were formed under equilibrating conditions. Assuming a planar amide function and a trans manner of addition of the mercapto group to the double bond (*Z* configuration), models indicate that the all-cis compound will probably be formed preferentially since all three groups can then be in a pseudoequatorial conformation.

We were interested in the conversion of the *N*-trifluoroacetyl DHA and DHP into the free enamines (**12**) by non-



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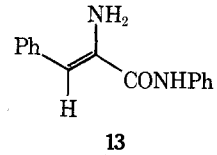

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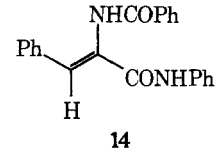

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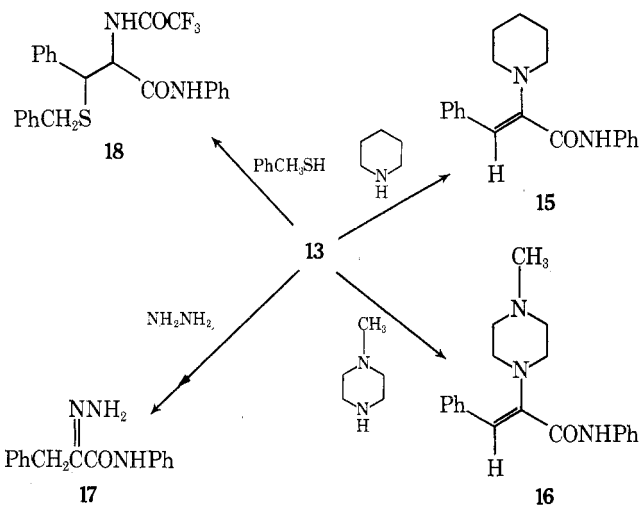
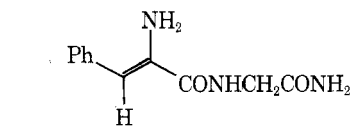
hydrolytic means so that further conversion to more complex dehydro peptides might be possible. In order to study the deblocking step, we elected to study the reactions of the anilide **5a** with various deblocking agents. It has been reported that sodium hydroxide and ammonium hydroxide,[15a] Amberlite IR-4B ion exchange resin,[15b] imidazole,[15c] various amines,[15d] and alcoholic sodium borohydride[15e] can be used to remove the trifluoroacetyl group. None of these reagents reacted with **5a** to any extent, as shown by thin layer chromatography (TLC) of the reaction mixtures. The blocking group was, however, removed by treatment of a solution of **5a** in THF with gaseous ammonia over a 15-h period and the crystalline enamine **13** was isolated in 74% yield. The structure of **13** was secured by elemental and spectral analyses and by its conversion to the benzamido anilide **14**, an authentic sample of which was prepared by treatment of the appropriate unsaturated azlactone with aniline. Acid-catalyzed hydrolysis of **13** to phenyl pyruvanilide was, surprisingly, very slow at room temperature. Some further surprising results were obtained when **13** was treated with piperidine and *N*-methylpiperidine. An exchange reaction occurred giving the α-piperidino- and α-piperazinocinnamanilides, **15** and **16**, respectively, in 80–90% yields. Hydrazine hydrate converted **13** into the hydrazone **17**, isolated in 33% yield. The anilide **13** also reacted with benzyl mercaptan giving the expected Michael addition product (**18**) in 85% yield. This reaction was, however,



very slow, requiring a large excess of mercaptan and the addition of triethylamine to the refluxing reaction mixture in order to drive it to completion.

Treatment of dehydro dipeptide amide (**6n**) and the dehydro tripeptide **7** with the NH_3–THF reagent gave the enamino dipeptide **19** and tripeptide ester **20** in good yields. These were stable crystalline compounds which



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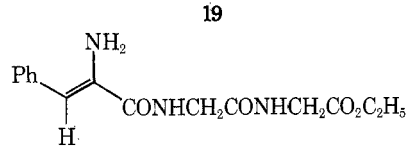

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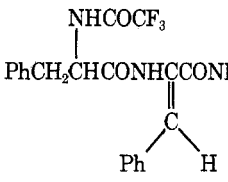
could be recrystallized from aqueous ethanol without significant loss of the enamine function by hydrolysis. It appears that DHP having N-terminal DHA, at least where the DHA is dehydrophenylalanine, are much less rapidly hydrolyzed under neutral conditions than might be expected.

Another aspect of the chemistry of the enamine system was the extreme lack of reactivity of **13** toward coupling with an "activated" acid. Attempts to couple **13** with acids using *N,N'*-dicyclohexylcarbodiimide, Woodward's reagent K, or by an isobutyl chlorocarbonate mixed anhydride procedure failed. No reaction at all occurred. As previously mentioned, **13** did react with benzoyl chloride to yield the amide **14**, so that treatment of **13** with *N*-trifluoroacetylphenylalanyl chloride[15b] gave the dehydro dipeptide derivative **21** in 41% yield, as expected. Similarly, the tetrapeptide derivative **22** was obtained in 57% yield from the ena-



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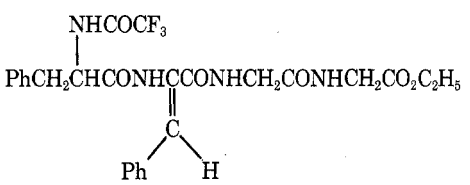

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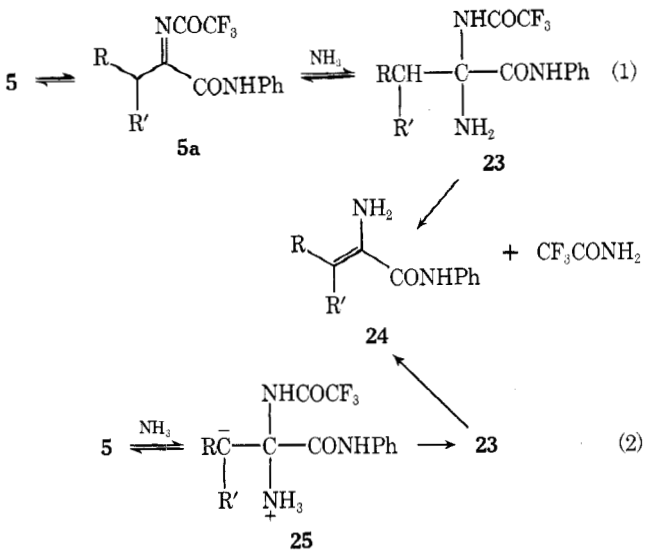
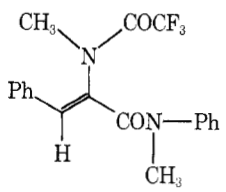
mino tripeptide **20**. No attempt was made to determine the optical purity of the single asymmetric centers in these compounds. It might be expected that the enamine nitrogen atom would be less nucleophilic than nitrogen in a similar saturated system owing to conjugation of the nonbonded electrons with the π electrons of the double bond. We might also predict that this effect would be enhanced in the dehydrophenylalanine system since the amino group is also conjugated through the double bond with the benzene ring. The amino groups of other less conjugated DHA might, consequently, be more reactive and more easily converted into dehydro peptides.

When the ammonia deblocking procedure and piperidine displacement reactions were applied to the other *N*-trifluoroacetyl DHA anilides, we found that only the phenylalanine derivative reacted cleanly.[16] We also found that the *saturated* anilide, *N*-trifluoroacetyl-DL-phenylalanine anilide, did not react with the NH_3–THF reagent. We interpreted these and our previous findings in terms of two possible deblocking mechanisms (Scheme I). Mechanism 1 requires that ammonia add to the tautomer **5a** giving an intermediate **23** which eliminates trifluoroacetamide to form the enamine **24**. This mechanism should be operable on any of the dehydro anilides (**5**) or peptides. It would seem also that the equilibrium **5** $\rightleftharpoons$ **5a** might have a smaller equilibrium constant in the case of phenylalanine owing to the favorable conjugation of the double bond in the enamine tautomer with the benzene ring. Mechanism 2 requires the formation of a carbanion (**25**) as the primary adduct, followed by proton transfer and elimination of trifluoroacetamide. This mechanism is consistent with the fact that the dehydrophenylalanine derivative is the only one found to react with ammonia, since the anion **25** should be stabilized more by the presence of a β-phenyl than by a β-alkyl group. This mechanism predicts that other anion stabilizing groups, such as carbonyl, should allow ammonolysis of *N*-trifluoroacetyl enamines. The fact that the *N,N'*-dimethyl derivative (**26**) of **13** does not react (TLC) with ammonia or piperidine, even under forcing conditions, might indicate that the imine tautomer (**5a**) is required for the reaction to

Scheme I



proceed. However, steric effects may play a role in this failure of **26** to react. Furthermore, the fact that benzyl mer-



captan added to the β-carbon atom of **5**, albeit slowly, rather than the α carbon is unexplained by either of these mechanisms. Perhaps the "softer" sulfur nucleophile is more likely to add to the "softer" β carbon,[17] while the "harder" nitrogen atom reacts more readily with the "harder" α carbon—its hardness being due to the electron-withdrawing effects of the adjacent trifluoroacetamido and carbonyl groups. We are in the process of studying further the reactions of *N*-acyl enamines.

Experimental Section

Instrumentation. All melting points were determined on a Nalge Model Y6 micro hot stage and are uncorrected. Infrared spectra (Nujol) were recorded on a Perkin-Elmer Model 257 or 631 recording spectrometer with polystyrene as the standard. The ^{1}H NMR spectra were recorded on a Varian HA-100 spectrometer using tetramethylsilane as internal or external standard, and ^{13}C NMR spectra were determined on a JEOL PFT-100 spectrometer with Me_4Si as internal standard; chemical shifts were obtained from the computer output. Optical rotations were obtained on a Perkin-Elmer Model 141 polarimeter. Elemental analyses were carried out by Atlantic Microlabs, Atlanta, Ga. Thin layer chromatography was carried out on Kodak ultraviolet-sensitive silica gel sheets, which were visualized by uv.

Materials. The α-amino acids and dipeptides were purchased from Sigma Chemical Co. and used as received.

General Procedure. 2-Trifluoromethyl 4-Substituted 3-Oxazolin-5-ones (2). A mixture of 0.1 mol of the α-amino acid and 32 ml (0.23 mol) of trifluoroacetic anhydride was refluxed (hot water bath) for 40 min. After removing the excess reagents, the residue was distilled at reduced pressure. All of the pseudoazlactones used in this work have been previously reported by Weygand[10] except **2a** and **2f. 2a:** 50% yield; bp 115–118 °C (0.4 Torr); ir (neat) 1805 (C=O), 1645 (C=N), 1175 cm^{-1} (C–F); NMR ($CDCl_3$) δ 3.85 (d, 2 H, J = 2.5 Hz, $PhCH_2$), 5.86 (m, 1 H, CF_3CH), 7.25 ppm (s, 5 H, C_6H_5); ^{19}F NMR ($CFCl_3$) 79.5383 ppm (d, CF_3CH). **2f:** 66% yield; bp 56–60 °C (12 Torr); ir (neat) 1810 (C=O), 1655 (C=N), 1170 cm^{-1} (C–F); NMR ($CDCl_3$) 1.35 (t, 3 H, J = 7.5 Hz, CH_3CH_2), 2.76 (2 q, 2 H, J = 7.5 and 2.1 Hz, CH_3CH_2), 6.26 ppm (m, 1 H, CF_3CH).

General Procedure. 2-Trifluoromethyl-4-(1-bromoalkyl)-3-oxazolin-5-ones (3). To a cold (ice bath) solution of 50 mmol of **2** in 90 ml of 1,2-dichloroethane was added 5 ml of a solution of 8.0 g (50 mmol) of bromine in 35 ml of 1,2-dichloroethane. A small sample was withdrawn from the reaction mixture, heated until decolorization occurred, and returned to the reaction flask. This process was frequently repreated two to three times to obtain decolorization of the reaction mixture. The remaining bromine solution was then added. After decolorization was complete, the solution was stirred for an additional 1 h, the solvent was removed in vacuo, and the residue was distilled under reduced pressure. The physical data for **3a,c,d** are given in Table I. Compounds **3b,e,f** were previously reported by Weygand.[10]

2-Trifluoromethyl-4-benzylidene-2-oxazolin-5-one (4a). A. Using Triethylamine (TEA). To a solution of 4.0 g (12.4 mmol) of **3a** in 300 ml of anhydrous ether was added over a 5-min period 1.71 ml (12.4 mmol) of TEA in 75 ml of ether. After 15 min the white precipitate was filtered (1.96 g, 87%) and the filtrate was concentrated to dryness in vacuo, giving 2.6 g of a solid residue. Crystallization from 6:1 isopropyl alcohol–water gave 2.3 g (77%) of crystalline **4a:** mp 102–103 °C; ir (Nujol) 1835, 1818, 1785 (C=O), 1670 (C=N), 1655 shoulder (C=C), 1170 cm^{-1} (C–F); NMR ($CDCl_3$) δ 7.34–7.60 (m, 4 H, vinyl H, 2 H meta, 1 H para), 8.0–8.18 ppm (m, 2 H, 2 H ortho).

Anal. Calcd for $C_{11}H_6NO_2F_3$: C, 54.80; H, 2.49; N, 5.81. Found: C, 54.78; H, 2.50; N, 5.90.

(*Z*)-α-Trifluoroacetamidocinnamic Acid (8a). To a solution of 1.0 g (3.1 mmol) of **3a** in 150 ml of ether was slowly added 0.427 ml (3.1 mmol) of TEA in 55 ml of anhydrous ether. After 15 min TEA·HCl was filtered (0.549 g, 95%) and the filtrate was concentrated in vacuo. The resulting white residue was dissolved in a 20:7 acetone–water solution. After 20 h at room temperature, the solvent was removed in vacuo and the solid residue (0.6 g) was crystallized from chloroform containing a few drops of hexane to give 0.503 g (63%) of **8a:** mp 196–199 °C; ir (Nujol) 3255 (NH), 1715 (CF_3C=O), 1700 (COOH), 1640 (C=C), 1175 cm^{-1} (C–F); ^{1}H NMR [$(CD_3)_2CO$] δ 7.30–7.48 (m, 3 H, 2 H meta, 1 H para), 7.50–7.66 (m, 2 H, 2 H ortho), 7.70 (s, 1 H, vinyl H), 9.47 and 9.71 ppm (2 broad s, 2 H, COOH, CF_3CONH); ^{13}C NMR [$(CD_3)_2CO$] δ 116.8 (CF_3), 124.6, 129.5, 130.6, 130.9, 133.8, 137.8 (aromatic and olefinic carbons), 157.6 (CF_3C=O), 165.21 (COOH).

Anal. Calcd for $C_{11}H_8NO_3F_3$: C, 51.00; H, 3.08; N, 5.40. Found: C, 50.92; H, 3.09; N, 5.51.

(*Z*)-Methyl α-Trifluoroacetamidocinnamate (8b). To a clear solution of 0.457 g (1.84 mmol) of **8** (R = H) in 15 ml of anhydrous ether was added 35 ml of ethereal diazomethane. After 10 min the excess diazomethane was removed under nitrogen and evaporation of the solvents in vacuo gave a clear oil which was crystallized from 3:1 diethyl ether–petroleum ether forming 350 mg (71%) of **8b,** mp 76–80 °C. An analytical sample, mp 79–80 °C, was recrystallized from methanol–water: ir (Nujol) 3200 (NH), 1730 ($COOCH_3$), 1700 (CF_3CO), 1635 (C=C), 1160 cm^{-1} (C–F); NMR ($CDCl_3$) δ 3.78 (s, 3 H, $COOCH_3$), 7.35 (s, 5 H, C_6H_5), 7.54 (s, 1 H, PhC**H**=), 8.04 ppm (broad s, 1 H, $NHCOCF_3$).

Anal. Calcd for $C_{12}N_{10}NO_3F_3$: C, 52.75; H, 3.67; N, 5.14. Found: C, 52.76; H, 3.55; N, 5.12.

2-Methoxy-2-trifluoromethyl-4-benzylidene-1,3-oxazolidin-5-one (9). A solution of 1.0 g (3.1 mmol) of **3a,** 0.43 ml (3.1 mmol) of TEA, and 6 ml of methanol in 110 ml diethyl ether was stirred at room temperature for 4 h. The reaction mixture was filtered, the filtrate was evaporated in vacuo, and the residue was chromatographed on a silica gel column using methylene chloride. The yellow solid (0.42 g) obtained was crystallized from CCl_4–petroleum ether to give 0.25 g (28%) of **9**: mp 126–128 °C; ir (Nujol) 3340 (NH), 1795 (C=O), 1665 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3 H, OCH_3), 5.56 (broad s, 1 H, NH), 6.46 (s, 1 H, vinyl H), 7.36 ppm (s, 5 H, C_6H_5); ^{19}F NMR ($CFCl_3$) 86.54 ppm (5, CH_3OCCF_3).

Anal. Calcd for $C_{12}H_{10}NO_3F_3$: C, 52.75; H, 3.66; N, 5.14. Found: C, 52.60; H, 3.73; N, 5.17.

General Procedure. *N*-Trifluoroacetyldehydroamino Acid Anilides (5). To a solution of 5 mmol of **3** in 50 ml of anhydrous ether was slowly added 0.92 ml (10.1 mmol) of aniline in 5 ml of ether. After standing at room temperature for 12 h, the aniline hydrobromide was filtered (0.87 g, 100%) and the filtrate was concentrated to 10 ml in vacuo. The insoluble anilide was filtered and further evaporation of the filtrate in vacuo gave a residue which, after trituration with ether, was dried in vacuo. The combined crops of **5** were crystallized from 4:1 methanol–water. The physical data for these anilides are given in Table II.

General Procedure. *N*-Trifluoroacetyldehydro Peptides (6) and Perhydro-1,4-thiazepin-5-ones (11). A solution of 3.0 mmol of the required amino ester hydrochloride in 100 ml of DMF and 0.835 ml (6 mmol) of triethylamine was stirred at room temperature for 45 min. To this solution was added, over a 5-min period, 3.0 mmol of **3**, dissolved in 20 ml of DMF. After stirring at room temperature for 24 h, the solvent was removed in vacuo and the semisolid residue was dissolved in 85 ml of ethyl acetate. The solution was washed with two 25-ml portions of water and once with 20 ml of 0.1 N HCl solution and dried (anhydrous $MgSO_4$) and the solvent was removed in vacuo. The residue was triturated with anhydrous ether and the insoluble product was collected and crystallized. If the residue was soluble in ether, it was directly crystallized from the appropriate solvents. The physical data for **6** and **11** are recorded in Tables III and IV, respectively.

***N*-Trifluoroacetyldehydrophenylalanylphenylalanine (6p).** To a solution of 0.512 g (3.1 mmol) of DL-phenylalanine and 0.65 g (6.2 mmol) of sodium carbonate in 25 ml of water was added 1.0 g (3.1 mmol) of **3a** dissolved in 30 ml of 1,2-dimethoxyethane. After 13 h at room temperature, the reaction mixture was filtered, the filtrate was concentrated in vacuo and diluted with ice water, and the pH was adjusted to 1 and concentrated HCl. This solution was extracted with two 30-ml portions of ether, and the combined extracts were dried (anhydrous $MgSO_4$) and concentrated in vacuo. The oily residue was crystallized from 3:1 chloroform–petroleum ether, giving 0.32 g (26%) of **6p**, mp 182–188.5 °C. An analytical sample was recrystallized from methanol–water: mp 188–190 °C; ir (Nujol) 3255 (NH), 2600–2750 (COOH), 1728 (CF_3C=O), 1700 (COOH), 1655 (C=C), 1628 (CONH), 1175 cm^{-1} (C–F).

Anal. Calcd for $C_{20}H_{17}F_3N_2O_4$: C, 59.00; H, 4.18; N, 6.88. Found: C, 59.02; H, 4.20; N, 6.87.

(*Z*)-Dehydrophenylalanine Anilide (13). Into one neck of a 50-ml three-necked round-bottomed flask, fitted with a drying tube and containing a solution of 1.70 g (5 mmol) of **5a** in 45 ml of THF, was bubbled ammonia for 15 h, the course of the reaction being followed by TLC ($CHCl_3$ elution). Evaporation of the solvent in vacuo gave a solid which was triturated with 20 ml of ether, and, after chilling, the insoluble solid was collected and dried giving 0.52 g of **13**, mp 118–124 °C. Concentration of the filtrate in vacuo followed by trituration of the residue with ether gave a second crop of **13**, 0.36 g, mp 121–126 °C, total yield 0.88 g (74%). An analytical sample was crystallized from 2:1 ethyl acetate–petroleum ether: mp 117–122 °C; ir (Nujol) 3390 (broad, NH), 1655 (C=C), 1625 cm^{-1} (CONHPh); ^1H NMR (Me_2SO-d_6) δ 5.06 (s, 2 H, NH_2, exchanged in D_2O), 6.12 (s, 1 H, vinyl H), 7.00–7.78 (m, 10 H, 2 C_6H_5), 10.01 ppm (s, 1 H, CONHPh); ^{13}C NMR (Me_2SO-d_6) δ 102.36 (PhCH=), 165.15 (C=O), 120.46, 123.57, 125.46, 127.84, 128.45, 136.86, 137.16, 138.93 (aromatic carbons and C=CNH_2).

Anal. Calcd for $C_{15}H_{14}N_2O$: C, 75.59; H, 5.88; N, 11.77. Found: C, 75.38; H, 5.93; N, 11.72.

(*Z*)-α-Benzamidocinnamic Acid Anilide (14). To a cold solution (ice bath) of 0.22 g (0.93 mmol) of **13** in 10 ml of ethyl acetate containing 0.2 ml (2.5 mmol) of pyridine was added 0.12 ml (1 mmol) of benzoyl chloride. After 1 h, the reaction mixture was allowed to come to room temperature for 4 h. The precipitated **14**, 0.151 g (49%), was filtered and dried in vacuo. Crystallization from methanol–water gave an analytical sample: mp 231–235 °C; ir (Nujol) 3280 (broad, NH), 1650 (C=C), 1600 cm^{-1} (CONHPh); NMR (Me_2SO-d_6) δ 6.95–8.14 (m, 16 H, 3 C_6H_5, vinyl H), 10.20 (broad, NH).

An authentic sample of **14** was prepared according to the procedure of Jansen et al.[18]

(*Z*)-2-(Piperidin-1-yl)cinnamic Acid Anilide (15). A solution of 1.06 g (3.17 mmol) of **13** and 0.32 ml (3.2 mmol) of piperidine in 25 ml of THF was stirred at room temperature for 18 h. After evaporation of the THF in vacuo, the solid residue was triturated with anhydrous ether and the insolubles were filtered, wt 0.76 g. A second crop of 0.13 g was obtained by the repetition of this procedure, total wt 0.89 g (92%) of **15**, mp 133–138 °C. An analytical sample, mp 138–140 °C, was crystallized from methanol: ir (Nujol) 3325 (NH), 1655–1660 (C=C), 1600 cm^{-1} (CONHPh); NMR (Me_2SO-d_6) δ 1.55 (broad s, 6 H, piperidine ring), 2.98 (broad s, 4 H, piperidine ring), 5.50 (s, 1 H, vinyl H), 6.90–7.69 (m, 10 H, 2 C_6H_5), 10.21 ppm (s, 1 H, NH).

Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.26; H, 7.27; N, 9.18.

(*Z*)-2-(4-Methylpiperidin-1-yl)cinnamic Acid Anilide (16). A solution of 1.19 g (3.57 mmol) of **13** and 0.41 ml (3.7 mmol) of *N*-methylpiperazine in 35 ml of THF was stirred at room temperature for 26 h. The solvent was evaporated in vacuo, giving a solid residue which was triturated with anhydrous ether and the insolubles were filtered, giving 0.96 g (84%) of **16** mp 168–176 °C. An anayltical sample was crystallized from ethyl acetate: mp 171.5–174.5 °C; ir (Nujol) 3220 (broad NH), 1650 (C=C), 1605 cm^{-1} (CONHPh); NMR (Me_2SO-d_6) δ 2.28 (s, 3 H, NCH_3), 2.48 (broad s, 4 H, piperidine ring), 3.08 (broad s, 4 H, piperidine ring), 5.62 (s, 1 H, vinyl H), 7.0–7.9 (m, 10 H, 2 C_6H_5), 10.2 ppm (s, 1 H, NH).

Anal. Calcd for $C_{20}H_{23}N_3O$: C, 74.74; H, 7.21; N, 13.07. Found: C, 74.55; H, 7.26; N, 13.11.

3-Phenylpyruvanilide Hydrazone (17). A solution of 1.16 g (3.37 mmol) of **13** and 0.18 ml of 95% hydrazine hydrate in 35 ml of THF was stirred at room temperature for 18 h. The solvent was removed in vacuo and the oily residue was dissolved in ether. After 24 h at 0 °C, the solution gave 0.146 g of **17** which was collected and dried in vacuo. Concentration of the filtrate, followed by trituration of the residue with 10 ml of ether, gave another 0.132 g of **17**: total wt 0.278 g (33%); mp 100–103 °C; ir (Nujol) 3220, 3305, 3340, 3400 (NH), 1650 (C=C), 1600 cm^{-1} (CONHPh); NMR (Me_2SO-d_6) δ 3.93 (s, 2 H, $PhCH_2$), 6.90–7.80 (m, 10 H, 2 C_6H_5), 7.49 (broad s, 2 H, NNH_2, exchanged with D_2O), 9.50 ppm (s, 1 H, NHPh).

Anal. Calcd for $C_{15}H_{15}N_3O$: C, 71.13; H, 5.97; N, 16.59. Found: C, 71.08; H, 5.72; N, 16.54.

***N*-Trifluoroacetyl-3-benzylthio-DL-phenylalanine Anilide (18).** A solution of 2.79 g (8.35 mmol) of **13** and 1.76 ml (15 mmol) of benzyl mercaptan in 100 ml of THF containing 5 drops of TEA was refluxed for 54 h. Since TLC showed the reaction to be incomplete, an additional 10 ml of benzyl mercaptan was added and the solution refluxed for a period of 10 days. The crude product was separated by column chromatography on silica gel, eluting first with *n*-hexane and then with 1:1 CH_2Cl_2–$CHCl_3$. The oily residue obtained was crystallized from 1:2 ether–petroleum ether, giving three crops of **18** totaling 3.24 g (85%), mp 118–126 °C. An analytical sample was recrystallized from the same solvent mixture: mp 126–128 °C; ir (Nujol) 3325, 3355 (NH), 1732 (CF_3CO), 1665 cm^{-1} (CONHPh); NMR ($CDCl_3$) δ 3.35 (d, 1 H, J = 14 Hz, $PhCHSCH_2Ph$), 3.68 (s, 2 H, $PhCH_2S$), 4.03 (d, 1 H, J = 14 Hz, $CF_3CONHCH$), 6.98–7.40 (m, 15 H, 3 C_6H_5), 8.09, 8.69 ppm (2 s, 2 H, $NHCOCF_3$, CONHPh).

Anal. Calcd for $C_{24}H_{21}N_2O_2SF_3$: C, 62.90; H, 4.59; N, 6.12. Found: C, 62.77; H, 4.64; N, 6.03.

(*Z*)-2-(*N*-Methyltrifluoroacetamido)cinnamic Acid *N*-Methylanilide (26). A mixture of 0.37 g (1.1 mmol) of **5a**, 1.0 g (7.25 mmol) of finely divided potassium carbonate, and 0.14 ml (2.23 mmol) of methyl iodide in 25 ml of DMF was stirred at room temperature for 7 h. The solvent was removed in vacuo, leaving a residue which was partitioned between ethyl acetate and water. The organic layer was washed with two 20-ml portions of water, dried (anhydrous $MgSO_4$), and concentrated in vacuo, giving an oily residue. Crystallization of the oil from ether–petroleum ether gave 0.31 g (77%) of the dimethyl compound (**26**): mp 129–131 °C; ir (Nujol) 1730 (CF_3 C=O), 1660 (C=C, CONHPh), 1170 cm^{-1} (C–F); NMR (Me_2SO-d_6) δ 2.74 [s, 3 H, $CON(CH_3)Ph$], 3.40 (s, 3 H, CH_3NCOCF_3), 6.40 (s, 1 H, vinyl H), 7.23–7.52 ppm (m, 10 H, 2 C_6H_5).

Anal. Calcd for $C_{19}H_{17}N_2O_2F_3$: C, 63.00; H, 4.70; N, 7.74. Found: C, 63.11; H, 4.75; N, 7.79.

Dehydrophenylalanylglycine Amide (19). Ammonia gas was bubbled for 2 days into a solution of 0.624 g (1.98 mmol) of **6n** in 40 ml of THF. Evaporation of the solvent in vacuo gave a solid residue which was triturated with ether and chilled, and the insoluble **19** (0.4 g) was collected, mp 121–135 °C. Crystallization from ethanol–water gave 0.32 g (73%) of **19**, mp 136–142 °C. An analytical sample was obtained from ethanol–water: mp 143–146 °C; ir (Nujol) 3180, 3325, 3385, 3500 (NH), 1660, 1645, 1610 (C=C, CONH); NMR (Me_2SO-d_6) δ 3.80 (d, 2 H, $NHCH_2CO$), 5.08 (s, 2 H, NH_2C=C, exchanged with D_2O), 6.00 (s, 1 H, vinyl H), 7.00–7.50 (m, 7 H, C_6H_5, $CONH_2$), 8.4 ppm (broad t, 1 H, $CONHCH_2$).

Anal. Calcd for $C_{11}H_{13}O_2N_3$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.22; H, 5.77; N, 19.20.

Dehydrophenylalanylglycylglycine Ethyl Ester (20). Ammonia gas was bubbled for 4 days into a solution of 0.921 g (2.3 mmol) of **7m** in 45 ml of THF. Evaporation of solvent in vacuo gave a white solid residue, which was triturated with 12 ml of anhydrous ether. After cooling to 5 °C, the insoluble **20** (0.45 g) was collected, mp 109–117 °C. The trituration process was repeated with 10 ml of ether, giving a total of 0.48 g (69%) of **20**. An analytical sample was crystallized from ethanol–water: mp 121–124 °C; ir (Nujol) 3245, 3330, 3420 (broad, NH), 1740 ($COOC_2H_5$), 1650, 1625 cm^{-1} (C=C, CONH); NMR (Me_2SO-d_6) δ 1.21 (t, 3 H, J = 7 Hz,

CH_3CH_2), 3.88 (d, 4 H, 2 $COCH_2NH$), 4.12 (q, 2 H, J = 7 Hz, CH_3CH_2), 5.06 (s, 2 H, $NH_2C{=}C$, exchanged with D_2O), 6.00 (s, 1 H, vinyl H), 7.05–7.52 (m, 5 H, C_6H_5), 8.27, 8.52 ppm (2 broad t, 2 H, $CONHCH_2$, both exchanged in D_2O).

Anal. Calcd for $C_{15}H_{19}N_3O_4$: C, 59.01; H, 6.27; N, 13.76. Found: C, 59.09; H, 6.29; N, 13.84.

***N*-Trifluoroacetylphenylalanyldehydrophenylalanine Anilide (21).** A solution of 0.38 g (1.6 mmol) of **13** and 0.39 g (1.4 mmol) of *N*-trifluoroacetylphenylalanyl chloride[15b] in 20 ml of THF was stirred at room temperature for 8 h. The solvent was evaporated in vacuo, the residue was dissolved in ethyl acetate, and the solution was washed with 10 ml of water and 15 ml of 5% $NaHCO_3$ solution. The organic layer was dried (anhydrous Na_2SO_4) and evaporated in vacuo leaving an oily residue. Crystallization from ethanol–water gave 0.294 g (44%) of **21**: mp 206–209 °C; ir (Nujol) 3200–3270 (broad, NH), 1700 (CF_3CO) 1660, 1635 (C=C, CONH), 1160 cm^{-1} (C–F); NMR (Me_2SO-d_6) δ 3.35 (m, 2 H, $PhCH_2$), 4.87 (m, 1 H, $PhCH_2CH$), 7.00–7.82 (m, 16 H, 3 C_6H_5, vinyl H), 9.83 (d, 1 H, $NHCOCF_3$), 10.00, 10.26 ppm (2 s, 2H, PhNHCO, CONHC=).

Anal Calcd for $C_{26}H_{22}N_3O_3F_3$: C, 64.75; H, 4.57; N, 8.73. Found: C, 64.82; H, 4.62; N, 8.78.

***N*-Trifluoroacetylphenylalanyldehydrophenylalanylglycylglycine Ethyl Ester (22).** A solution of 0.21 (0.69 mmol) of **20** and 0.21 g (0.75 mmol) of *N*-trifluoroacetylphenylalanyl chloride[15b] in 20 ml of THF was stirred at room temperature for 13 h. The solvent was evaporated in vacuo, giving a residue which was crystallized from an ethanol–water solution in two crops, total wt 0.215 g (57%), mp 183–200 °C. Three recrystallizations from ethanol–water gave an analytical sample: mp 200–206 °C; ir (Nujol) 3250 (NH), 1752 ($COOC_2H_5$), 1710 (CF_3CO), 1675, 1660, 1620 (C=C, CONH), 1175 cm^{-1} (C–F); NMR (Me_2SO-d_6) δ 1.21 (t, 3 H, J = 7.0 Hz, CH_3CH_2), 3.38 (m, 2 H, $PhCH_2$), 4.39 (m, 4 H, 2 CH_2NH), 4.11 (q, 2 H, J = 7 Hz, CH_3CH_2OCO), 4.86 (broad, 1 H, $PhCH_2CH$), 7.07–7.65 (m, 11 H, 2 C_6H_5, vinyl H), 8.20 (broad, 2 H, 2 $NHCH_2$), 9.70 (broad d, 1 H, $NHCOCF_3$), 10.08 ppm (s, 1 H, NHC=).

Anal. Calcd for $C_{26}H_{27}F_3N_4O_6$: C, 56.93; H, 4.92; N, 10.22. Found: C, 56.52; H, 4.90; N, 9.97.

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Supplementary Material Available. Ir and NMR data for **3a, 3c, 3d,** and **5a–e** and NMR data for **6a–p** and **10a–f** (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Presented at the Fourth American Peptide Symposium, New York, N.Y., June 1975.
(2) Abstracted from the Ph.D. Dissertation of Edward G. Breitholle, Feb 1975.
(3) N. Leonard and R. Ning, *J. Org. Chem.*, **32,** 677 (1967); (b) M. Ohta, H. Kurita, M. Masaki, and Y. Chigira, *Bull. Chem. Soc. Jpn.*, **41,** 2758 (1968); (c) M. Ohta, C. Shin, and M. Masaki, *J. Org. Chem.*, **32,** 1860 (1967); (d) J. Yoshimura, C. Shin, and K. Nanjo, *Tetrahedron Lett.*, 521 (1974); (e) U. Schmidt, A. Perco, and E. Ohler, *Chem. Ber.*, **107,** 2816 (1974).
(4) (a) B. W. Bycroft, *Nature (London)*, **224,** 595 (1969); (b) E. Gross and J. L. Morell, *J. Am. Chem. Soc.*, **93,** 4634 (1971); (c) E. Gross et al., *Hoppe-Seyler's Z. Physiol. Chem.*, **354,** 799, 802, 805, 807, 810 (1973).
(5) M. Bodanszky and D. Perlman, *Science*, **163,** 352 (1969); A. I. Scott, J. Cheney, C. Moore, J. Raleigh, and D. Youne, *J. Chem. Soc., Chem. Commun.*, 47 (1974); J. E. Baldwin, S. Haber, and J. Kitchin, *ibid.*, 790 (1973).
(6) L. Zervas and N. Ferderigos, *Isr. J. Chem.*, **12,** 139 (1974).
(7) (a) D. H. Rich, C. Mabuni, J. Mathiaparanam, and J. Grant, *J. Chem. Soc., Chem. Commun.*, 897 (1974); (b) D. Rich and J. Tam, *Tetrahedron Lett.*, 211 (1975).
(8) (a) C. H. Stammer and J. Riordan, *J. Org. Chem.*, **39,** 654 (1974); (b) E. G. Breitholle and C. H. Stammer, *Tetrahedron Lett.*, 2381 (1975).
(9) Morin has investigated the oxidation of azlactones also; cf. R. B. Morin, E. M. Gordon, T. McGrath, and R. Shuman, *Tetrahedron Lett.*, 2159 (1973); R. B. Morin and E. M. Gordon, *ibid.*, 2163 (1973).
(10) F. Weygand and W. Steglich, *Angew. Chem.*, **73,** 433 (1961); (b) F. Weygand, W. Steglich, and H. Tanner, *Justus Liebigs Ann. Chem.*, **658,** 129 (1962); F. Weygand, W. Steglich, D. Mayer, and W. vonPhilipsborn, *Chem. Ber.*, **97,** 2023 (1964).
(11) Many of the unsaturated azlactones (**4**) give black, tarry products when even a slight excess of base is used.
(12) Recently dehydropeptides have been ruled out as intermediates in penicillin biosynthesis; cf. F. Huang, J. A. Chan, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Am. Chem. Soc.*, **97,** 3858 (1975).
(13) E. Gross and L. J. Morell, *J. Am. Chem. Soc.*, **93,** 4634 (1971).
(14) (a) N. Leonard and G. Wilson, *J. Am. Chem. Soc.*, **86,** 5307 (1964); (b) N. Leonard and R. Ning, *J. Org. Chem.*, **31,** 3928 (1966); N. Leonard and R. Ning, *ibid.*, **32,** 677 (1967); (c) R. Mitchell and M. Benn, *Can. J. Chem.*, **50,** 2195 (1972).
(15) (a) F. Weygand and E. Casendes, *Angew. Chem.*, **64,** 136 (1952); (b) E. Schallenberg and M. Calvin, *J. Am. Chem. Soc.*, **77,** 2779 (1955); (c) C. E. Stauffer, *J. Am. Chem. Soc.*, **96,** 2489 (1974); (d) M. Khosla, personal communications; (e) F. Weygand and E. Frauendorfer, *Chem. Ber.*, **103,** 2437 (1970).
(16) We also found that the analogous *N*-benzoyl compound (**14**) reacted slowly and under forcing conditions with *N*-methylpiperidine to give **16** in 6% isolated yield.
(17) R. G. Pearson, *J. Chem. Educ.*, **45,** 581, 643 (1968).
(18) A. Jansen and R. Robinson, *Monatsh. Chem.*, **98,** 1017 (1967).